Cellular imaging in drug discovery

Paul Lang, Karen Yeow, Anthony Nichols and Alexander Scheer

Abstract | Traditional screening paradigms often focus on single targets. To facilitate drug discovery in the more complex physiological environment of a cell or organism, powerful cellular imaging systems have been developed. The emergence of these detection technologies allows the quantitative analysis of cellular events and visualization of relevant cellular phenotypes. Cellular imaging facilitates the integration of complex biology into the screening process, and addresses both high-content and high-throughput needs. This review describes how cellular imaging technologies contribute to the drug discovery process.

Charge-coupled device camera
A charge-coupled device is a silicon chip whose surface is divided into light-sensitive pixels. When a photon hits a pixel it registers as an electric charge that can be counted. With large pixel arrays and high sensitivity, CCDs can create high-resolution images and are often incorporated into cameras to take such pictures.

Department of Molecular Screening & Cellular Pharmacology, Serono Pharmaceutical Research Institute, 14 chemin des Aulx, 1228 Plan-les-Ouates, Switzerland.
Correspondence to P.L. or A.S.
e-mails: Paul.Lang@serono.com; Alexander.Scheer@serono.com
doi:10.1038/nrd2008

Despite the remarkable scientific advances over the past few decades, little positive effect has been seen on the drug discovery industry. The increased investment in R&D has not yet paid off: there has been a steady decline in the number of drugs against new targets reaching the marketplace[1]. Analysts point to various reasons for this decrease in productivity. One surprising observation is that this decline coincides with the advent of combinatorial chemistry and high-throughput screening (HTS)[2]. The target-based approach — which is based on the assumption that disruption of a single gene or molecular mechanism is a key event in disease ontogeny — promised increased screening capacities and rational drug discovery, and has replaced the more traditional physiology-based approach that emerged more than two decades ago.

The perceived failure of this model for drug discovery has sparked many debates about how to improve it. A common view is that disease or biology-relevant screens and models should be brought into the drug discovery process much earlier in an attempt to decrease the current drug attrition rate. Within this context, modern cell biology provides a means through which we can model different aspects of disease biology. Primary human cells can be used as *in vitro* systems in various therapeutic areas, and such biologically relevant cell assays are becoming increasingly recognized as screening tools that are robust and amenable to automation/HTS[3]. However, the realization of such a strategy requires practical tools that enable the implementation of a more biology-driven approach to drug discovery.

Cellular imaging is emerging as a crucial tool that would enable the integration of biological complexity into drug discovery. However, cellular imaging has always been a largely descriptive science, and in its early days such techniques were solely amenable to small-scale experimental samples, typically gathering data from 10–1,000 cells at a time. In addition, such systems required constant monitoring by highly skilled personnel, particularly when handling data extracted from digital images, limiting the use of imaging technologies to target identification or mechanism of action studies.

We define cellular imaging as the use of a system/technology capable of visualizing a cell population, single cell or subcellular structures, applied in combination with image-analysis tools. Such detection systems include (but are not limited to) microscopes, fluorescence macro-confocal detectors and fluorometric imaging plate readers (FLIPR) used with charge-coupled device (CCD) cameras. These systems generate a two-dimensional pixel array of information (a digital image) extracted from a particular biological event or tissue type. Various image-analysis tools have been developed to process the information in the digital image into meaningful parameters.

The ideal imaging system would allow high-resolution analysis of single cells, high throughput and kinetic studies on live cells, and would be linked to efficient data storage and compression systems as well as user-friendly image-analysis programs. Although we have yet to attain this ideal, today's imaging technologies are robust enough to be used in the pharmaceutical industry. Modern imaging platforms allow plates to be read quickly, enabling an increased number of conditions to be tested in the same experiment. In addition to the reading of plates, the automation of image acquisition, processing and analysis with user-friendly interfaces greatly encouraged the integration of modern cellular imaging. Equally important (but beyond the scope of this review) have been the advances in labelling and detection materials. A survey published in 2005 indicated that cellular imaging is also now heavily used in secondary compound screening, and in compound progression[4]. Indeed, cell screening is expanding in all discovery organizations. Roche Pharmaceuticals increased cellular

screening from 20% in 1998 to 50% in 2003 (REF. 5). Serono cellular screening efforts increased from 5% in 1998 to 50% in 2005. Clearly, cellular imaging is here to stay: it has been a mainstay in the technology platforms of most pharmaceutical companies for the past 3–6 years, and its use is likely to increase in the future.

Cellular imaging can be used in all stages of target-based drug discovery that involve the study of cells, including target discovery, drug screening in cell-based assays, early safety evaluation, mode-of-action studies and *in vivo* studies to monitor cell fate.

In this review, we present various cellular imaging technologies and describe how they can be applied in the drug discovery process. We also discuss how cellular imaging has contributed to drug discovery and development from the delivery of new targets to the progression of lead compounds and clinical candidates, and how it could affect the efficiency of these processes in the future.

Target identification and validation

A target is usually a single gene, protein or molecular event that is thought to be involved in the aetiology of a disease. Cellular imaging has long been a tool used for target discovery, traditionally in the analysis of fixed tissue or cell samples. Visible-light microscopy allows non-invasive three-dimensional imaging of live and fixed cells at the sub-micron scale with high specificity, and is routinely used in laboratories to investigate the role of targets in cellular phenotypes. Nowadays, fluorescence microscopy has become one of the most popular imaging tools as its success was catalysed by the advent of fluorescent proteins and tags. Among the many types of fluorescence microscopy, confocal microscopy is now widely used in the pharmaceutical industry because this fairly simple-to-use method allows the imaging of cells in optical sections at high resolution, and allows multiplexing of the interposed images. Laser-scanning microscopes work in a similar manner to confocals, but are much faster in performing optical sectioning.

The use of fluorescent proteins has revolutionized cell biology by enabling the behaviour of proteins to be tracked in live cells (BOX 1). We are beginning to exploit a wide range of fluorescent proteins using multicolour fluorescence microscopy to study diverse biological processes within cells and uncover new targets. Green fluorescent proteins, for instance, have been used for just such purposes. This has allowed the real-time analysis of the localization of two RAS GTPases (RAS and RAP) after epidermal growth factor (EGF) stimulation. This study assessed GFP–RBD (RAS-binding domain) localization in COS cells overexpressing the small GTPase of interest and GFP–RBD[6]. Such applications of fluorescent proteins are of great interest to researchers in the field of cancer biology, because it can be used as a read-out of post-translational processing, a pathway controlled by druggable cancer targets such as farnesyl transferases. However, resolution is currently limited by the diffraction of light to 200 nm[7]. It is often impossible to distinguish between single objects and those that are simply in close proximity. Therefore, proteins that seem to be co-localized by fluorescence microscopy might actually be separated by a considerable molecular distance. Fluorescence resonance energy transfer (FRET) technology counters this problem through detection of the transfer of excitation energy from donor to fluorophore acceptor molecules, allowing spatial resolution of molecular proximity in the scale of nanometres. This energy transfer can be captured using confocal or multiphoton microscopy. Temporal resolution of protein–protein interactions can be achieved using fluorescence lifetime imaging (FLIM)[8]. FLIM monitors localized changes in the fluorescence lifetime of probes and is well suited for analysis of dynamic changes within living cells. The application of FLIM in parallel with FRET can provide strong evidence for physical interactions between proteins with a high spatio-temporal resolution. Indeed, FLIM has been used in combination with a two-photon excitation system to monitor the kinetics of drug–DNA interactions in living cells[9]. The docking of the anticancer drug topotecan (Hycamptin; GlaxoSmithKline) onto its binding site consisting of the topoisomerase I–DNA complex was visualized in live breast tumour cells using these techniques. The FLIM–FRET combination has also been used to demonstrate that the low-density lipoprotein receptor-related protein (LRP) is a novel substrate for the β-secretase b-site APP-cleaving enzyme 1 (BACE1)[10].

Another imaging technique applied in target discovery is flow cytometry. This technique is based on the principle that individual particles (such as cells) suspended in a stream of liquid are individually interrogated by a focused light source[11]. The most common system for labelling is the use of antibodies coupled to fluorescent moieties. Such systems can gather multi-parametric data from as many as 100,000 cells per second. Flow cytometry can also be used to isolate specific cells from a mixed population, making it a valuable tool for analysis of rare events. Flow cytometry has been successfully applied to identify novel tumour-associated cell-surface markers as potential targets for biotherapeutics[12]. The expression of specific target antigens on CD34-positive/CD38-negative cells were analysed from a population of leukaemic stem cells from a patient with various myeloid malignancies, using multi-colour flow cytometry[13]. This study showed that target antigens (including CD13, CD33 and CD44) are expressed in neoplastic stem cells from various myeloid neoplasms, suggesting that these stem cells display a similar phenotype. Not all of these targets are expressed on all the neoplastic cells, and so specific combinations of targeted antibodies will be necessary to eliminate all of the tumour cells expressing various combinations of these markers.

With the advent of the human genome project in combination with the extended use of new validation tools such as small interfering RNA (siRNA), target discovery teams are facing the challenge of assessing a large number of targets simultaneously. Nowadays, modern cellular imaging techniques are capable of quantifying cellular phenotypic changes (FIG. 1), allowing a detailed study of the role of a particular target in *in vitro* models that reflect the disease[14–18], and the functional analysis of target and

Laser-scanning microscopy
A laser beam passing a light source aperture is focused into a small focal volume in a fluorescent specimen. The mixture of emitted fluorescent and reflected laser light from the illuminated spot is then separated so that the laser light passes through and reflects the fluorescent light on to the detection apparatus.

Fluorescence resonance energy transfer
(FRET). FRET is a distance-dependent interaction between the electronically excited states of two dyes that can be used to measure biological phenomena that produce changes in molecular proximity.

Multiphoton microscopy
A standard technique for three-dimensional imaging of thick fluorescent specimens.

Box 1 | **Fluorescent reporter probes**

During the past decade, the development of fluorescent proteins from marine organisms has revolutionized the study of protein localization and function in living cells. These fluorescent proteins provide convenient markers for gene expression and protein targeting *in vivo*, from single cells to whole organisms[112]. Genetically encoded proteins expressed endogenously within cells as fusion proteins with fluorescent markers are more likely to retain their native localization patterns and functional activities[113]. Today, many options exist for the fluorescent tagging or labelling of proteins. The most well-known fluorescent probe, isolated from the jellyfish *Aqueorea victoria*, is known as green fluorescent protein (GFP). Spectral variants of GFP exist in different colours, including blue, cyan and yellow-green. The discovery of red fluorescent proteins from Anthozoan corals has expanded the colour repertoire[112]. These red and far-red fluorescent proteins are of special interest, as eukaryotic cells and tissues display reduced autofluorescence at these wavelengths. Longer excitation wavelengths also mean less damage to proteins and DNA, and less photobleaching. These fluorescent proteins are members of a homologous protein family, ranging in size from 25 to 30 kDa, and form internal chromophores that do not require cofactors or substrates to fluoresce[114].Their high extinction coefficients and high quantum yields mean that these proteins fluoresce very brightly. However, a disadvantage is that the size of such protein tags could alter cell physiology or protein function. In addition, costly patent issues hamper the use of many fluorescent proteins for large-scale screening purposes. In addition to the GFPs, the development of many other fluorescent systems is underway. The recent developments in photoactivatable fluorescent proteins, capable of pronounced changes in their spectral properties in response to irradiation with specific wavelengths of light, hold much promise for applications in the kinetic microscopy of living cells[115].

pathway modulation in living cells. Fluorescence-based microscopy techniques are versatile because of the availability of flexible resolution options and the possibility of using them at different magnifications (TABLE 1).

The power of such an approach has recently been highlighted in the discovery of new targets for cancer[15]. Morphological changes were induced in five human cell types (four cancer cell lines and one primary human cell) by a set of 107 small molecules (encompassing four scaffolds known to inhibit protein kinases). Cells were treated with various concentrations of compounds for 24 hours and stained for visualization of the nuclei, Golgi apparatus and microtubules. Changes in such cellular processes provide unbiased analysis of the morphological effects induced by these chemicals, and has been validated by analysis of known pharmacologically active compounds from ten different mechanism of action classes. Image analysis using an epifluorescence microscope led the authors to characterize each compound according to a morphological signature. They identified hydroxy-PP (a hydroxylated analogue of the pyrrolopyrimidine Src kinase inhibitor, PP2), a compound that showed a morphological signature distinct from a known inhibitor of almost identical structure. Further molecular analysis revealed that this compound was targeting cellular NADPH-dependent carbonyl oxidoreductase 1 (CBR1). This hypothesis was further validated by a study using siRNA, which uncovered the function of CBR1 in serum-mediated apoptosis.

Microscopy platforms that enable the assessment of phenotypic changes that are difficult to quantify, such as neurite outgrowth[19] and cell migration[20,21], allow not only the identification of targets through phenotypic screening but also have the potential to shed light on their role in complex cellular systems. In one study, confluent monolayers of epithelial cells in 384-well plates were subjected to scratch wounding prior to treatment with compounds to screen for compounds that affect wound healing and cell migration[18]. The actin cytoskeleton was visualized by phalloidin staining, and an image of each well was captured using automated microscopy. Compounds affecting wound healing were grouped according to their effects on cell phenotype, and further profiled against seven secondary assays. These efforts resulted in the identification of a new RHO kinase inhibitor with a novel scaffold that has potential for optimization. In addition, unexpected molecules active in the process of wound healing, such as cyclin-dependent kinase (CDK) inhibitors and the kinase inhibitor Rottlerin were discovered, leading to the identification of new targets that could be important for wound healing.

Automated microscopy platforms can be run in medium- or high-throughput mode to screen thousands of cDNAs, siRNAs, aptamers, proteins and antibodies in cells in target discovery[22]. Recently, a chemical genetic study was performed in parallel with a genome-wide RNA interference (RNAi) screen to identify cytokinesis inhibitors and their targets[23]. This assay principle was based on the fact that cells that undergo mitosis normally but fail to undergo cytokinesis acquire two nuclei. To detect binucleate cells, an amine-reactive ester was used to stain the cytoplasm, and Hoechst dye was used to visualize the DNA. These inexpensive reagents allowed cost-efficient screening of about 20,000 RNAi and 50,000 small molecules using automated fluorescence microscopy. This fast-track approach led to the identification of 214 protein targets important for cytokinesis, including 25 previously uncharacterized proteins. Furthermore, by comparative phenotypic analysis, the authors successfully identified a class of small molecules with phenotypic effects on cells that had no RNAi counterpart, possibly because of a gain-of-function effect. In this case, cellular imaging allowed the quantification of phenotypes that were previously unquantifiable, facilitating the identification of novel targets.

Perhaps the most revolutionary feature of modern cellular imaging in target discovery is to provide a multidimensional aspect for each experiment performed. The potential for measurement of multiple parameters allows for the analysis of biological responses towards different stimuli[24,25]. For instance, such information could provide clues as to how cancer cells exploit signalling pathways for continued growth, and thereby reveal potential new targets for therapeutic intervention. This multiplexing approach has been successfully applied for profiling cancer cell signalling networks in patients[26]. In this study, flow cytometry analysis allowed the detection of up to 13 activated signalling molecules per CD33-positive myeloid cell isolated from patients. The authors performed single-cell measurements to detect changes in signalling patterns of phospho-protein networks, enabling both the classification of patients as well as the identification of targets. In the field of immunology, profiling of multiple cytokines or intracellular signalling molecules can be performed in the same well, allowing researchers to identify targets involved in the T-cell response. Tools such as cytometric

Epifluorescence microscopy
This method uses a type of microscope that has a very bright light source. Ultraviolet, blue, yellow or red light from the light source is then used to energize the specimen, which will then re-emit light at various wavelengths to be viewed by the observer.

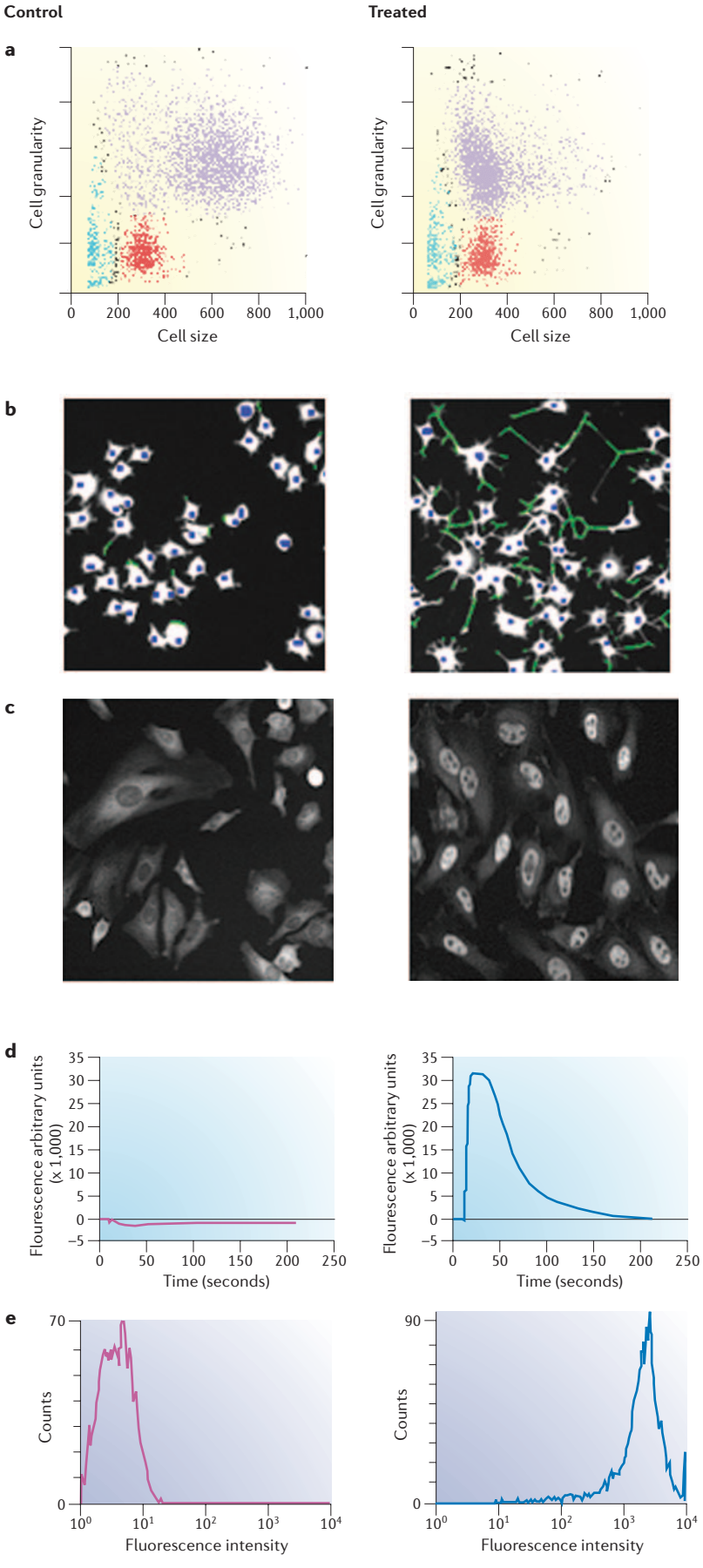


Figure 1 | **Cellular imaging applications.** Cellular imaging platforms enable the detection of cellular phenotypic changes in a cell population. **a** | Addition of a protein on peripheral blood mononuclear cells followed by flow cytometry analysis allows the detection of neutrophil cell-shape change (purple), whereas lymphocyte shape is unaffected (blue). **b** | Phenotypic changes such as neurite outgrowth can also be quantified using automated microscopy platforms. Following addition of nerve growth factor (NGF), a rat pheochromocytoma cell line (NS-1) exhibits neurite outgrowth (highlighted in green) that can be detected using fluorescence staining and automatically quantified by powerful cell-pattern-recognition software. **c** | Screening for subcellular changes is also possible using cellular imaging platforms. Following stimulation, translocation of an intracellular protein from cytosol to nucleus is detected and quantified by means of fluorescently labelled antibodies, such as phosphorylated extracellular signal-regulated kinase antibodies in HeLa cells[132]. **d** | Fluorometric imaging plate readers monitor the kinetics of intracellular calcium release as a read-out of a generic second-messenger. After stimulation, cells release calcium from the endoplasmic reticulum into the cytosol that in turn will react with a calcium-sensitive fluorescent dye. Graphs show calcium release in G-protein-coupled receptor (GPCR)-transfected Chinese hamster ovary cells. The graph on the right depicts the kinetics of calcium flux after stimulation. **e** | Flow cytometry analysis of surface antigen upregulation in human blood cells can be used as clinical biomarkers. Here, stimulation of whole blood with lipopolysaccharide (LPS) leads to CD11b upregulation on neutrophils.

bead arrays (beads coated with antibodies against the target of choice)[27] when used in combination with flow cytometry or fluorescence macro-confocal imaging allow researchers to assess how disease phenotypes can be modulated. For instance, interleukin-25 (IL-25), a newly identified T_H2 cytokine capable of amplifying allergic inflammation, has been shown to induce secretions of a subset of cytokines (such as monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1α (MIP1α), IL-6 and IL-8) in eosinophils[28]. Such multiplexed read-outs are useful tools for the assessment of multiple targets involved in a particular disease.

Compound screening

The combined advances in the industrialization of cell biology and the introduction of cellular imaging screening devices has allowed us to define the concept of parallel screening (FIG. 2). In this context, cellular imaging platforms confer distinct advantages compared with other types of read-outs (such as non-image-based plate readers)[29,30]: for example, imaging of a whole area using a laser-scanning device (as is the case for one cellular imaging platform) is much faster than moving individual cells in front of a detector (as for non-imaging plate readers). In addition to speed, cellular imagers provide flexible resolution options. A cell population can therefore be monitored using low resolution[31,32], whereas changes in subcellular structures can be recorded using high resolution[5,33–36]. Screening for cell-proliferation inhibitors in cancer biology could be

Table 1 | **Cellular imaging techniques commonly used for *in vitro* applications**

Technique	Resolution	Applications	Therapeutic area
Low-magnification fluorescence microscopy	500 µm–1mm	Entire cell populations: phenotype, proliferation, toxicity, migration and angiogenesis	Oncology and cardiovascular disease
Medium-magnification fluorescence microscopy	10–50 µm	Subpopulation analysis: individual cell phenotype, for example, neurite outgrowth or cell differentiation	Neurology, metabolic diseases and cardiovascular disease
High-magnification fluorescence microscopy	1 µm	Intracellular molecular events: nuclear translocation, micronucleus formation and mitosis	Oncology, inflammation and neurology
Flow cytometry/laser scanning devices	1–30 µm	Subpopulation analysis or intracellular modifications: surface marker regulation, whole blood cells, phenotypic changes and proliferation	Autoimmunity, inflammation and cardiovascular disease

taken as a case study. Here, cellular imaging techniques can be used at low magnification/low resolution to monitor compound potency on the whole-cell population of a well. By contrast, determination of which stage in the cell cycle a particular compound exerts its effects requires the same machine to be used in high-magnification/high-resolution mode. The use of high and low resolution can therefore be exploited to provide complementary information during compound screening[37].

Many screening groups have been using cellular imaging for HTS purposes for more than 10 years, particularly for screening G-protein-coupled receptor (GPCR) modulators using FLIPR[38]. A generic read-out for GPCR functional activity is calcium flux, a second messenger released as a result of receptor stimulation. FLIPR is popular for these purposes as it allows the monitoring of calcium flux kinetics in whole cells through the use of calcium-sensitive dyes. Usually, calcium flux is assessed in cell lines stably transfected with the GPCR of interest, such as adrenergic receptors[39], melanocortin receptors[40], neurokinin receptors[41] or purinergic P2Y receptors[42]. Similarly, FLIPR has been used for screening voltage-gated sodium channels[43] or glycine transporters in overexpressed cellular systems through the use of dyes to measure membrane potential[44].

The advent of automation in combination with sophisticated yet user-friendly image analysis software resulted in another generation of imaging platforms able to provide a wealth of information from single cells. The recent commercial success of automated imaging platforms from companies such as Cellomics[45–47], TTP[48], Evotec[49], Molecular Devices (see Further information) and GE-Healthcare[50,51] is largely due to their easy-to-use features, minimal supervision requirements and user-friendly software. TABLE 2 summarizes the contents of some of these platforms.

These new technologies provide flexibility in terms of resolution. When used at low resolution, they are able to quantify phenotypic changes across entire cell populations (such as changes in cell number and cell shape). In high-resolution mode, subcellular changes can be quantified (such as protein translocation across subcellular compartments). In addition, data-analysis software provides researchers with a wide choice of parameters for analysis, including number of neurites per cell, average neurite length and number of branch points. Companies such as Cellomics also provide their customers with optimized reagents for their platforms (even optimized cell lines), which significantly reduces assay development time. Therefore, certain applications, such as neurite outgrowth[19] or nuclear translocation assays[5] (which are traditionally run in low throughput), can now be run in medium-throughput-screening mode.

Currently, most cellular imaging devices are used for secondary screening or lead optimization[52,53], as they are able to provide multiple types of information on compound selectivity in a cellular context using their multiplexing capacities. For example, a cell-based assay enabling detection and quantification of nuclear factor-κB (NF-κB), p38 and c-JUN translocation in response to inflammatory stimuli was successfully used to profile compound selectivity between these signalling pathways[54]. Multiplexing of read-outs in this case enabled researchers to obtain selectivity information in the same experiment that measured compound activity.

However, implementation of these assays onto cell-imaging-screening platforms confers several challenges. The first is to properly validate these assays, especially in the case of those that have not been pre-optimized by the vendors. For example, optimization of staining conditions is often difficult, as many commercially available antibodies have not been validated for use under these conditions. Such 'home-made' protocols can still be validated and run, but often with a lower throughput and under the supervision of a highly skilled person.

The second challenge is to combine the biological relevance of cellular imaging assays with a reasonable throughput. Currently, most robotic platforms have been set up to deal with simpler assays, mostly biochemical ones. The challenge is to automate more complex cell-based assays with higher throughput, while maintaining the necessary robustness and integrity of the living cells. Most of the time, these assays are run in 96-well format (although many can be run in 384-well plates). The time needed for processing each plate depends on the desired resolution and the number of cells per well. Typically, a 96-well plate can be processed in 5 minutes for a low-resolution application, whereas a high-resolution screen can take up to 120 minutes per plate when detecting rare events (P.L., K.Y., A.N. and A.S; unpublished observations).

The third challenge is the handling and analysis of data. Typically, multiple images are collected from each well at different magnifications, and processed by the integrated system software that analyses each image, recognizes appropriate cell patterns, and extracts measurements relevant to the biological application. This process results in the generation of a vast amount of numerical data, which needs to be stored in terabyte-sized databases (except in the case of laser scanner-generated data, which require less space). There is much pressure to find the appropriate data storage and management solutions, as raw images need to be archived for 6–10 years[4].

Finally, the last challenge lies with the choice of read-out: the chosen parameters have to be robust, with high signal-to-noise ratios, and reflect the desired biology as closely as possible. Indeed, image algorithms can provide scientists with various parameters related to an observed biological event. The challenge will be to select the correct parameters with which to mine the data.

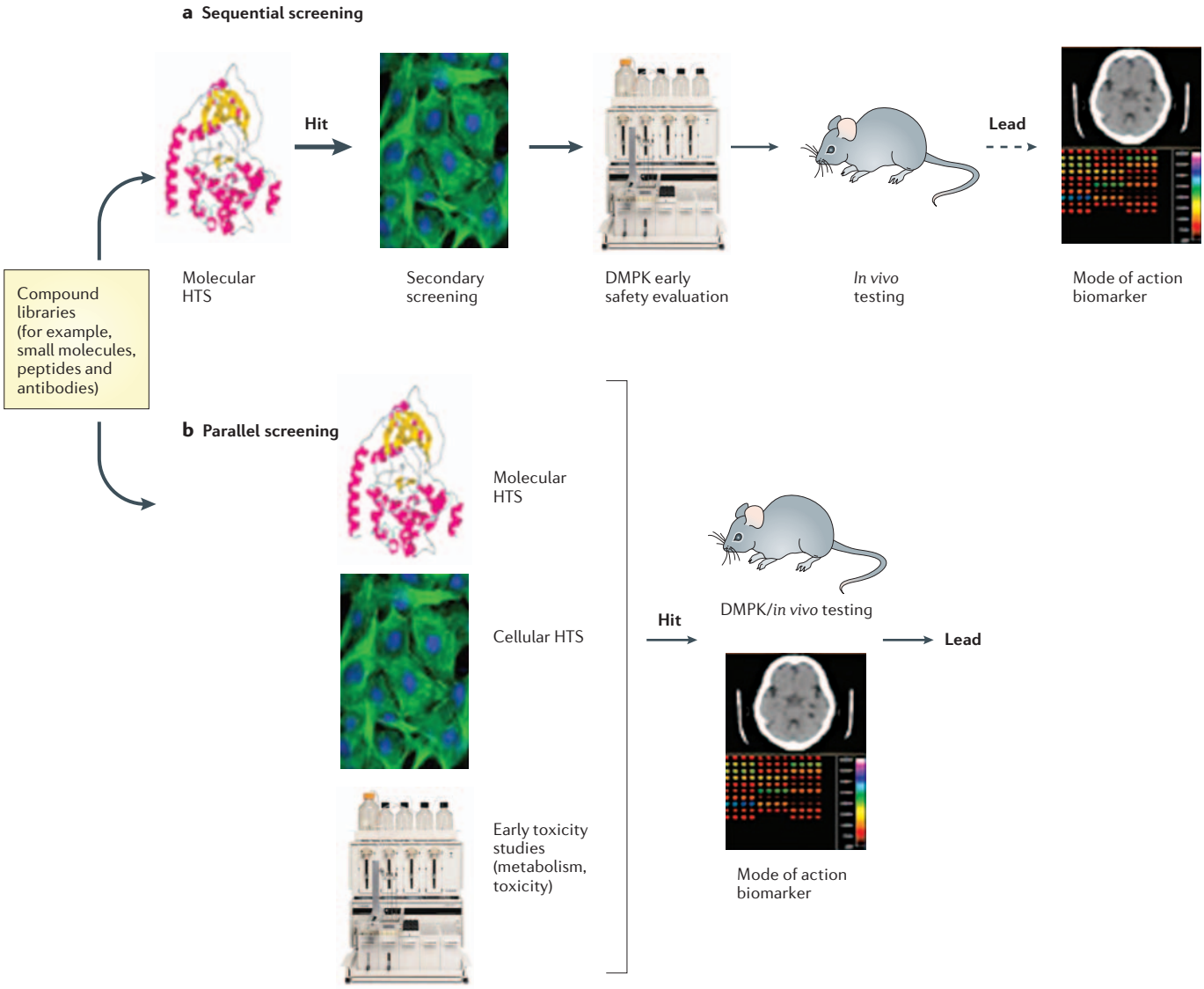


Figure 2 | **Sequential versus parallel screening.** Target-based drug discovery is organized as a series of sequential steps that enable characterization of molecular entities. **a** | Shows the sequential approach in which high-throughput screening (HTS) against single targets generates hits that are subsequently tested in functional secondary assays, followed by preclinical pharmacokinetics (PKs), absorption, distribution, metabolism, excretion and toxicity (ADMET), and *in vivo* studies. The resulting lead is then tested in Phase I–III trials and biomarkers are developed to enable studies of the compound's mode of action in humans. **b** | The application of cellular imaging could reshape the process, resulting in a more parallel screening approach. Molecular entities would be screened using high-content cellular assays that allow the simultaneous assessment of compound potency and toxicity. Where appropriate, screening could also be performed in parallel, enabling selection of compounds with different mechanisms of action and early rejection of those with toxic effects, thereby enriching the quality of the hits. After *in vivo* testing, active hits can be transferred to cellular imaging platforms for biomarker/mechanism of action studies.

Table 2 | **Most widely used automated cellular imaging platforms in industry in 2005***

Company	Brand name	Detection system	Plate handling?	CCD cameras?	Data storage?	Image-analysis software?	Assay-ready kits?	Throughput
Cellomics	ArrayScan HCS	Automated fluorescent microscope	Yes	Yes	Yes: data point linked to the cell image from which it is generated	Yes	Yes	Medium (<50,000 data points per day)
TTP LabTech	Acumen Explorer	Laser scanning fluorescence microplate cytometer	No	No	Yes: the absence of an image-processing stage allows the reduction of data files to 50kb in screening mode	Yes	No	Medium (<50,000 data points per day)
Evotec Technologies	Opera	Automated confocal microscopy	Yes	Yes: images are stored in a separate file space	Yes: images are stored in a separate file space	Yes	No	High (>1 million data points per day)
Molecular Devices	Discovery I	Automated fluorescent microscopy	Yes	Yes	Yes: data point linked to the cell image from which it is generated	Yes	No	Medium (<50,000 data points per day)
GE Healthcare	INCell Analyzer 3000	Automated confocal microscopy	Yes	Yes	Yes	Yes	Yes	High (>1 million data points per day)

*At least one of these platforms is currently used in multiple discovery sites of the top 15 pharmaceutical companies, as well as in many biotechnology companies and academic research centres. CCD, charge-coupled device.

Screening using cellular imaging is still in its infancy, as many hurdles need to be overcome before we can tap its full potential. In order to fully adapt it for HTS, systems with higher resolution, faster imaging and automation capacities, more flexible configurations and more reliable data-mining algorithms are needed. Although more and more assays are becoming robust, cellular imaging technologies still remain complex and costly. For example, bulk reagents, including antibodies and fluorescent probes, currently account for 31% of the operating budget of a cellular imaging platform, whereas 11% of the budget is dedicated to the purchase of new software[4]. However, despite the current limitations, cellular imaging techniques are very promising screening tools, as discussed below.

Drug discovery often focuses on molecular events that can be detected in cells or that affect cell–cell contacts. For example, in a search for compounds affecting cell–cell interaction, a series of gap junction inhibitors were identified by screening several hundred thousand compounds, using an automated cell imager[55]. This assay was run in 384-well format, and the use of live cells and the reading time per plate (43 minutes) limited screening campaigns to 12 plates per day. This led to the identification of selective gap-junction blockers useful for preventing the propagation of injury during ischaemia. In a similar strategy, the translocation of signalling molecules from one subcellular compartment to another was analysed in high-throughput mode[56,57]. In another example, FLIPR was used to identify small-molecule AKT pathway inhibitors in cells. The use of a 96-well format allowed medium- to high-throughput (5,000 compounds per day) screening campaigns. The selected hits were then further studied using enzymatic assays or high-content screening systems, resulting in the identification of several classes of translocation inhibitors with various modes of action (kinase activity inhibitors, modulators of protein–protein interactions and other pathway inhibitors)[56].

Currently, the use of cell lines is prevalent throughout the screening process. However, these are often derived from transformed cells, and although such cell lines have an improved proliferative capacity, they often display aberrant genetic and functional characteristics. The closer a cultured cell system matches the cells in human target tissues, the more likely it is that results obtained from such systems will be predictive of efficacy and safety *in vivo*. However, these cells are often limited in quantity and are difficult to obtain, and for certain tissue types (such as human neuronal cells) tissue is simply not available. Therefore, the use of primary cells is generally restricted to use at later stages of drug development. However, various alternatives are being explored to enable the use of primary cells in high-throughput mode. For instance, the concept of conditional immortalization provides the ability to switch off the effect of immortalizing oncogenes once the appropriate number of cells has been generated[58]. This strategy has been used to generate screenable quantities of myogenic, hepatic and neural cells[59]. Embryonic stem cells (ESCs) also provide an attractive alternative, which might allow the development of predictive screening assays that can deliver higher-quality leads[60].

Today, the stem-cell field is already benefiting from cellular imaging, such as for monitoring stem-cell fate *in vivo* (BOX 2). However, the application of mammalian stem cells in screening is still in its infancy, limited by robustness issues such as inappropriate cell differentiation and insufficient biomass. *In vitro* differentiation of totipotent stem cells can result in many cell types, and selecting particular cells of interest (such as neurons)

Box 2 | **Cellular imaging and cell therapies**

There is great promise in the use of cells as a means of delivering therapeutics, or as a therapy themselves. In the early 1990s, clinical immunotherapy trials were performed in which *ex-vivo* interleukin-2 (IL-2)-activated lymphocytes were transfused into patients with metastatic renal-cell carcinoma[116] in order to activate the patients' natural killer cell activity. Today, there is hope that the use of cell therapy for transplantation[117–119], treating myocardial infarction[120,121], severe autoimmune disease[122,123] or Alzheimer's disease[124,125] will be successful. However, non-invasive techniques need to be designed to monitor the fate of these cells following *in vivo* administration.

Modern cell biology in combination with state-of-the-art imaging techniques has triggered a revolution in whole-organism studies. Aside from *in vivo* studies, new generations of cellular therapeutics such as those based on neuronal progenitor cells or stem cells are already in early-stage clinical trials[126,127]. Cellular imaging has not only been crucial in the purification of stem cells, but is also essential for tracking the fate of these cells when transplanted into patients. Currently, cells are labelled *ex vivo* using markers such as BrdU, green fluorescent protein or LacZ before injection, and analysis of such cells entails their removal from the body. The use of magnetic resonance imaging (MRI) provides the possibility for non-invasive whole-body imaging with a resolution of 25–50 µm, which is approaching the resolution of a single cell. To this end, several different contrast agents have been developed based on the concept of loading cells with superparamagnetic compounds such as superparamagnetic iron oxide particles (SPIO)[128,129]. Ferumoxides such as SPIO and a liposome agent that facilitates cell loading — protamine sulphate — have been FDA-approved for labelling a variety of cell types without adverse short- or long-term effects on cell viability, proliferation or differentiation[129]. A recent study assessed the feasibility of labelling human neural stem cells with SPIO and tracking them by MRI after transplantation into a patient with open brain trauma[130]. The labelled cells could be tracked non-invasively up to 7 days post-surgery using MRI, and were shown to successfully populate the border zone of the damaged tissue around the lesion.

Ideally, imaging technology used for tracking transplanted cells would have single-cell-resolution capacities, and would permit quantification of the exact number of cells at a given anatomical location. In addition, tracking of such cells should be feasible over periods of months to years, as clinical trials require long-term follow-up studies. Although many imaging technologies could theoretically be used to monitor the *in vivo* fate of stem cells (that is, ultrasound, high-energy photon imaging or MRI), further work needs to be done to optimize these techniques for clinical use[131]. Nevertheless, it is likely that cellular labelling and imaging technologies will have a major role in advancing cell therapy in the future.

depends on the expression of marker proteins that can be exploited for the separation process. Here, cellular imaging techniques are perfectly adapted to the selection and isolation of specific cell populations for subsequent screening. Fluorescence-activated cell sorting (FACS) can be used to select the appropriate mammalian ESC derivatives, such as macrophages[61] (for example, by selecting for the IL-1 receptor) or endothelial cells[62]. *In vitro* differentiation protocols for murine ESC are becoming robust enough to be applied for screening purposes[63]. These cells can also be modified to incorporate reporter genes so that agonist or antagonist compounds that interact with the target protein can be readily identified by HTS[60]. Murine ESC are also elegant tools for assessment of the teratogenic and embryotoxic potential of drugs and chemicals[64]. The multiplexing capacity of flow cytometers and automated microscopy platforms enables the screening of mixed populations of differentiated ESC, therefore avoiding multiple selection runs. Indeed, the use of multiple but specific neuronal differentiation protocols for human stem cells in parallel could lead to the generation of oligodendrocytes, astrocytes and neurons in the same well[65]. Subpopulation identification is therefore essential, and screening of cell subpopulations using cellular imaging can yield a vast amount of data from only a few thousand cells per well. This approach could be very useful for evaluating compound mode of action, or to improve optimization of leads.

Fluorescence-activated cell sorter
(FACS). A FACS is a machine that can rapidly separate cells in suspension on the basis of size and the colour of their fluorescence.

Early safety evaluation

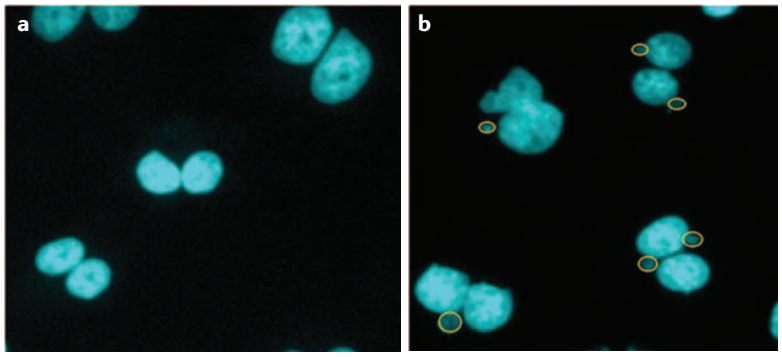
Of the numerous drug candidates that fail *en route* towards becoming approved as drugs, approximately 30% fail because of toxicity and clinical safety[66]. The current approach to identifying new drugs is to first achieve potency and biological activity before the new drug candidate is evaluated for toxicity in animals. The requirement for large quantities of compound for toxicity studies necessitates a complicated scale-up process. The large quantities needed restrict experimental analysis and throughput[67], and a lack of *in vitro* predictive tools for toxicity means that these studies are not carried out until the preclinical stage. Therefore, any failure occurring late in development increases the cost of drug discovery. The current focus is to improve preclinical administration, distribution, metabolism, excretion and toxicity (ADMET) studies[68], using predictive approaches such as toxicogenomics[69]. However, the understanding and prediction of the molecular basis of toxicology remains a major challenge.

The use of cellular imaging systems could offer distinct advantages in studies of ADMET and drug–drug interactions[70], providing an attractive complement to classical toxicology studies with biologically relevant *in vitro* tests performed at an early stage of drug development. The higher throughput, lower costs and lower quantities of compounds needed compared with classical *in vivo*-based ADMET assays[71] render imaging techniques highly suitable. In parallel with the assessment of their biological activity, toxicity testing of compounds in cells will help to define whether the cellular activity observed is related to target inhibition or to a compound-mediated toxic effect. The use of cellular imaging, along with its multiplexing capacities, enables the simultaneous assessment of desired on-target effects versus off-target toxic effects. A recent study described the use of automated microscopic platforms to quantify the nuclear translocation of various signalling molecules activating IL-1β expression (such as c-JUN, p38 and NF-κB). Specificity of selective small-molecule inhibitors against each of these targets was assessed by quantifying inhibition of nuclear translocation, while parallel analysis of nuclear staining intensity, total number of cells per well, nuclear shape and staining intensity ruled out compound-induced cell toxicity[54].

This process will not replace classical *in vivo* toxicology experiments, but rather will narrow down the selection of molecules by better characterizing them *in vitro*, enabling a greater chance of success in *in vivo* studies. In addition, it will permit a more detailed analysis of subtle, sub-lethal cellular phenotype changes, such as changes in cell shape or metabolism, which cannot be assessed using classical toxicology studies. Multiplexing several read-outs in the same well using hepatocytes (the classical cellular model for toxicology) could shed light on the mechanism of toxicity caused by various compounds.

Box 3 | ***In vitro* micronucleus assay**

The micronucleus assay (a system for detecting genotoxic agents that modify chromosome structure and segregation) can now be used as part of a series of genotoxicity tests required by regulatory agencies prior to drug approval. Micronuclei are pieces of damaged chromosomes or entire chromosomes that have failed to become included in daughter nuclei during cell division, and are therefore a convenient measure of genotoxicity. Today, the most commonly used assay to quantify micronucleus formation is the *in vivo* mammalian erythrocyte precursor assay. This assay is low throughput, requires large quantities of compound (gram scale), requires specialized staff, and is both labour- and time-intensive. By contrast, an *in vitro* micronucleus assay using cultures of established mammalian cell lines can overcome most of the hurdles described above. Briefly, cultured cells are treated for 24 hours in the absence (**a**) or presence (**b**) of compounds and scored for micronucleus induction using fluorescent dyes (see figure below). Micronuclei are circled in yellow and nuclei are depicted in blue. Automated cellular imaging platforms allow quantification of such phenotypes using a medium- or high-throughput format. As far less of a compound is required for such an assay, *in vitro* micronucleus formation can be assessed much earlier on in the drug discovery pipeline, making structure–genotoxicity relationships a possible means for compound characterization.



One could imagine assessing in parallel the dose-dependent effects of a compound on many cellular parameters, such as apoptosis (using caspase antibodies), cell number (nuclear staining) and DNA damage (TUNEL). Such *in vitro* experiments will enable us to understand whether toxicity is related to the chemical nature of the compound (facilitating the choice of another class of compounds) or to the molecular target itself.

The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis. Currently, the genotoxicity of compounds is assessed using the chromosomal aberration assay (*in vivo* micronucleus assay)[72]. This test uses bone-marrow-derived cells from compound-treated rodents to assess chromosomal content by means of immunostaining and manual scoring. Although biologically relevant, the disadvantages of this assay are its low throughput, high costs related to the amount of compound needed for testing and lack of accurate quantification. The classical chromosome aberration test requires several hundred milligrams of lead compound for testing and the turnaround time for the results is 40–70 days. An image-based *in vitro* micronucleus assay has been developed as an early test for genotoxicity (BOX 3). The results obtained correlate well with those obtained from chromosomal aberration assays[73].The cost per compound for testing using the *in vitro* micronucleus assay is less than €3,000, compared with more than €10,000 per compound using its *in vivo* counterpart (contract research organization quotes). The *in vitro* assay requires only 1–5 milligrams of compound for testing, provides the benefits of increased throughput and lower cost, and can be used to generate quantifiable dose–response data. The concept of the *in vitro* micronucleus assay has been around for several years[74]. But the real breakthrough now is the use of cellular imaging technology to quantify micronucleus formation in a high-content, high-throughput format. This assay is now becoming a mainstay in modern toxicology platforms, and is currently used by various pharmaceutical companies. The implementation of such technology does not abrogate use of the classical 'comet' or chromosomal aberration assays; rather, it enables us to assess earlier and with a higher throughput the potential effect of these compounds *in vitro*, bringing better characterized leads for testing in *in vivo* models.

Overall, cellular imaging confers a systematic approach to the study of pathways that can be modified by a given compound[75], allowing better prediction of the tissues most likely to be affected. In the future, the integration of experimental and predictive cellular data in combination with functional read-outs should facilitate the decision to stop or continue the development of specific molecules much earlier[76].

Early clinical development

Mode of action and biomarkers. Regulatory authorities are making increasing demands on drug developers to better characterize the mode of action of drugs and to reduce the risk of adverse drug reactions occurring in the clinic[77]. There is much pressure on preclinical drug development to provide certain key studies, such as bioanalytical assays capable of measuring the efficacy of drugs in a biological milieu, and proof-of-concept studies to understand their modes of action[78–80]. The application of more sensitive analytical methods, and advances in proteomics[81,82] and human genetics[83], have led to the identification of relevant biomarkers that will enable researchers to identify and characterize suitable patient populations for specific drugs, bringing personalized medicine one step closer[84–88].

Cellular imaging systems with multiplexed read-outs to test drug dose–response relationships, and pharmacokinetics on live cells might complement the information obtained from transcriptional and proteomic analyses, enabling a better selection of biomarkers for use in the clinic[89,90]. For instance, biomarkers such as CD4, in combination with analysis of viral loads by flow cytometry, have been instrumental to fast-track drug development and approval in HIV medicine[91].

High-content cellular imaging has recently been used to identify potential mechanisms of action of uncharacterized drugs[92]. Automated microscopy was applied to assess whether a set of compounds could affect the biology of a cell line of interest, and whether profiles of drug activity could be generated. The authors tested a set of compounds on HeLa cells (a human cancer cell line), including well-characterized molecules, as well as compounds with unknown modes of action. Treated cells were stained with various fluorescent probes that

TUNEL
Terminal deoxynucleotidyl transferase-mediated dUTP nick-end-labelling is used for quantifying apoptosis at the single-cell-level based on labelling of free 3′-OH terminals that occur as a result of DNA strand breaks.

covered a range of cellular phenotypes, and a set of descriptors was used for each probe to capture the information. Using advanced bioinformatics and statistics, a titration-invariant similarity score was developed that led to the classification of compounds based on biological outcomes rather than chemical similarity. Not only did this approach successfully categorize known, blinded drugs, it also revealed possible mechanisms of action for two previously uncharacterized ones.

A more thorough understanding of the molecular mechanism by which a drug modulates a specific target would also lead to the development of appropriate bioassays for clinical studies. As the tissue of choice is often blood, flow cytometry has been the mainstay for more than 20 years. Flow cytometers are now used routinely for biological marker assays, such as the analysis of cytokine expression[93–95], lymphocyte phenotyping[96] and cell-cycle analysis[97]. With the evolution of new technology for detection of novel antigens and signalling molecules[98], flow cytometry will be increasingly used for these purposes.

The ultimate goal is to deliver biomarkers to measure drug efficacy in the preclinical and clinical stages of development. The extension of cellular imaging techniques to *in vivo* studies[99,100] will be of immense benefit towards understanding drug action, enabling a more seamless transition from the laboratory to the clinic.

Determining* in vivo *drug efficacy using cellular imaging. Certain therapeutic areas such as cancer biology are already reaping the benefits of the use of cellular imaging techniques, for example, in monitoring cancer cell fate in live animals. Fluorescent proteins such as GFP have been used to monitor cell fate *in vivo*, through the injection of cancer cell lines stably transfected with GFP into animals[101]. Such studies using surgical orthotopic implantation of GFP-transfected Chinese hamster ovary cells in animals demonstrated that single metastatic cells could be detected in fresh tissues[102]. GFP-transfected cells can also be detected in live animals using real-time intravital microscopy at the single-cell level[103]. However, the use of such GFP models is limited by tissue density, which hampers fluorescence detection[101].

Bioluminescence imaging (BLI) is currently being used to the same end. Bioluminescence refers to the conversion of chemical energy into visible light, such as through the action of luciferase. Luciferase activity *in vivo* is based on the transmission of light through tissues that is detected by low-light imaging devices based on highly sensitive CCD cameras[104]. BLI is a highly quantitative technique that can be used to screen cancer inhibitors *in vivo* for their capacity to modulate a particular target. In one example, a transgenic mouse expressing a fusion protein between luciferase and the transcription factor E2F1[105] was engineered. Activation of this transcription factor is the result of one of the most common signalling pathway alterations found in human cancers: the inactivation of the retinoblastoma pathway. When injected with platelet-derived growth factor (PDGF, which activates the nuclear-binding activity of E2F1 (REF. 106)), the mice developed oligodendrogliomas that were detectable by BLI in the live animals. The image intensity correlated with tumour size as measured by histological techniques. Development of the tumours over time could also be measured using BLI (via the increase in photon emission from the brain area over a 5-day period). More importantly, PDGF receptor inhibitors resulted in a substantial decrease in light emission over time.

Another promising cellular imaging methodology is quantum dot bead technology. Quantum dots are minute, light-emitting particles with unique optical and electronic properties[107,108]. They have generated much excitement as superior tags for cellular imaging when combined with fluorescence scanning microscopy, thanks to the improved spectroscopic properties of the bead particles in comparison with organic dyes or fluorescent proteins. Calculations based on the optical properties of tissue suggest that the use of near-infrared-emitting quantum dots would allow the detection and resolution of 10–100 labelled cells. Quantum dots can be conjugated to various moieties such as DNA oligonucleotides, aptamers or antibodies. A convincing proof-of-concept study showed that quantum dots coupled to an antibody could recognize prostate-specific membrane antigen (PSMA), which is expressed at the surface of prostate cancer cells. These quantum dot probes were injected *in vivo* into C4-2 tumour xenografts maintained in athymic nude mice and the PSMA-positive cells could be detected by epifluorescence microscopy. Finally, when anti-PSMA-coated quantum dots were injected into animals bearing C4-2 tumour xenographs, prostate tumour cells could be visualized in live animals using spectral imaging. These technologies could soon see widespread use in the pharmaceutical industry for assessing the effects of compounds in similar *in vivo* systems.

Future challenges

Cellular imaging techniques are currently of most interest to the pharmaceutical industry for target discovery, lead optimization, *in vitro* toxicology and compound profiling[4]. So far, less attention has been given to the potential applications of cellular imaging for primary screening and for the study of cell fate in live animals. Indeed, several factors limit the extensive use of cellular imaging in primary screening (TABLE 3), such as the high cost of reagents, complex assay development and data-management issues[4]. In particular, cellular imaging is based on staining techniques, which involve many steps not amenable to automation. For example, centrifugation steps, precise fixation/permeabilization times, as well as numerous aspiration and dispensing procedures, are not HTS compatible. Future improvements might come from the development of automation-friendly reagents, such as commercial antibodies validated for imaging (stable for long periods of time at room temperature), or all-in-one fixation-permeabilization solutions. A combination of higher-resolution imaging systems and more user-friendly reagents will greatly facilitate the use of cellular imaging in primary screening.

Despite the pitfalls, the prevalence of cellular imaging as a drug discovery tool has increased the throughput

Intravital microscopy
The observation of cell fate in a living organism by the detection of green fluorescent protein.

Table 3 | **Current limitations of cellular imaging**

Drug R&D stage	Measure	Required throughput	Running costs	Limitations
Target identification	Phenotype	Medium/high	Low	• Some phenotypes are difficult to quantify in a robust manner • Assay development can take a long period of time
Primary screening (on target)	Subcellular events	Medium/high	High (antibodies/dyes)	• Automation of such assays might be extremely difficult in HTS mode • Complex screening procedures
Secondary compound screening	IC_{50} value against target/phenotype	Low/medium	High (multiplexing)	• Complex assay development and screening procedures • Multiplexing approaches require in-depth knowledge of data treatment
Mode-of-action studies	Subcellular modification	Low	High (multiplexing)	• Complex assay development and screening procedures • Multiplexing approaches require in-depth knowledge of data treatment
Biomarker	Cell surface marker	Low	Low/medium	• Flow cytometry still requires highly specialized personnel
In vivo cellular tracking	Cell fate	Low	High	• Technology still in its infancy. Requires combination of cell biology, imaging and *in vivo* skills

HTS, high-throughput screening.

of discovery platforms in pharmaceutical and biotechnology companies. The average number of routinely run screens incorporating cellular imaging in large pharmaceutical companies was estimated to be 6.8 screens in 2005 and is expected to increase to 10.4 screens in 2006 (REF. 4). In addition to screening relevant cell lines, cellular imaging will also facilitate the use of human primary cells and stem cells in assays. As biomass generation from primary cells is not equivalent to that of cell lines, the number of primary cells used per well should ideally be kept low (that is, hundreds of cells per well), as compared with higher numbers of cells from cell lines (at least thousands of cells per well) for performing large screening campaigns. The use of new platforms with higher resolution and multiplexing capacities will successfully exploit this limited biomass for large screening campaigns.

Another consideration is that many of the current phenotypic analyses and read-outs are performed on fixed cells. The use of live cells will enable a more dynamic analysis of the activity of drugs, facilitating kinetic analysis and toxicology studies in a 'real-time' manner. New imaging systems are beginning to appear on the market, including systems that can record real-time changes in cell morphology and physiology through a classical, visible light microscope without the addition of dyes or antibodies.

The implementation of cellular imaging in drug screening (in particular primary screening) brings tremendous challenges in quality control of the screens, as well as in data analysis. Even in the simplest of assays, there is the choice of a myriad of parameters for possible analysis; the challenge is to select the right one(s). Which parameter is the most relevant when assessing the effect of thousands of compounds on a neurite outgrowth assay. The same image can provide data on branch points, average number of neurites per cell, cell number or neurite length. The most realistic solution would be to take into account several relevant parameters for data quality control and selection of positives. This necessitates that biologists join forces with computer scientists and statisticians to assess the best program to use for exploiting the data. Such software packages are starting to become available to the industry[109] and are beginning to be implemented in this final crucial stage of the screening process.

In addition to measuring individual parameters, cellular imaging technologies can be promising tools for studying cell systems biology to understand physiology and disease at a whole-cell level. In this approach, a complex network of cell types, treatments and read-outs are analysed by machine-learning algorithms. The aim is to discriminate as many disease-modulating agents and drugs as possible while taking a minimal set of measurements[110]. This methodology requires that relevant information be selected by processing large amounts of data into computer models of regulatory networks. For instance, multidimensional flow cytometry was used to record 11 intracellular proteins and phospholipids in individual human peripheral blood cells subjected to nine treatments[111]. These data were processed using machine-learning algorithms and the system was able to automatically identify most of the previously reported signalling relationships while also predicting new pathways, which were then verified experimentally. Cellular imaging combined with powerful computing techniques could therefore revolutionize our understanding of the complexity of the mode of action of drugs, as well as our understanding of the dysfunctions of diseased cells.

How could cellular imaging affect the process of compound progression within the pipeline? Structure–activity relationships are commonly used by medicinal chemists to improve small-molecule properties[1]. Cellular imaging, when combined with systems biology, could promote a more biology-driven environment for

compound progression[110], therefore compelling us to think in terms of structure–phenotype relationships, or structure–pathway modulation relationships.

Overall, the most important effect of cellular imaging is to provide new ways of assessing the activity of our compounds. Whether it is to assess all the targets of the druggable genome by phenotypic screening, or by providing high-content screening capability, cellular imaging will have a profound influence on the manner in which we run our early drug discovery programmes. Cellular imaging should not be viewed as a tool to simply speed up the drug discovery process, but rather one that can maximize the value of each pharmaceutical company's treasure chest of compounds. Time will tell whether this approach will lead to the discovery of new drugs.

1. Hood, L. & Perlmutter, R. M. The impact of systems approaches on biological problems in drug discovery. *Nature Biotechnol.* **22**, 1215–1217 (2004).
2. Sams-Dodd, F. Target-based drug discovery: is something wrong? *Drug Discov. Today* **10**, 139–147 (2005).
3. Besson, D., Yeow, K., Lang, P. & Scheer, A. HTS and cellular biology at Serono. *Curr. Drug Discov.* 29–32 (2003).
4. Comley, J. High content screening: emerging importance of novel reagents/probes and pathway analysis. *Drug Discov. World* **6**, 31–54 (2005).
5. Ramm, P. Image-based screening: a technology in transition. *Curr. Opin. Biotechnol.* **16**, 41–48 (2005).
This review describes the advantages and disadvantages of using cellular imaging technologies in screening, and provides clues of what future cellular imaging systems requirements are for HTS purposes.
6. Bivona, T. G. & Philips, M. R. Analysis of Ras and Rap activation in living cells using fluorescent Ras binding domains. *Methods* **37**, 138–145 (2005).
7. Voss, T. C., Demarco, I. A. & Day, R. N. Quantitative imaging of protein interactions in the cell nucleus. *Biotechniques* **38**, 413–424 (2005).
8. Sekar, R. B. & Periasamy, A. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. *J. Cell Biol.* **160**, 629–633 (2003).
9. Errington, R. J. *et al.* Advanced microscopy solutions for monitoring the kinetics and dynamics of drug–DNA targeting in living cells. *Adv. Drug Deliv. Rev.* **57**, 153–167 (2005).
10. von Arnim, C. A. *et al.* The low density lipoprotein receptor-related protein (LRP) is a novel β-secretase (BACE1) substrate. *J. Biol. Chem.* **280**, 17777–17785 (2005).
11. Herzenberg, L. A. *et al.* The history and future of the fluorescence activated cell sorter and flow cytometry: a view from Stanford. *Clin. Chem.* **48**, 1819–1827 (2002).
12. Geuijen, C. A. *et al.* Affinity ranking of antibodies using flow cytometry: application in antibody phage display-based target discovery. *J. Immunol. Methods* **302**, 68–77 (2005).
13. Florian, S. *et al.* Detection of molecular targets on the surface of CD34 + /CD38− stem cells in various myeloid malignancies. *Leuk. Lymphoma* **47**, 207–222 (2006).
14. Heinemann, A. *et al.* Basophil responses to chemokines are regulated by both sequential and cooperative receptor signaling. *J. Immunol.* **165**, 7224–7233 (2000).
15. Tanaka, M. *et al.* An unbiased cell morphology-based screen for new, biologically active small molecules. *PLoS Biol.* **3**, e128 (2005).
16. Miller, S. C. & Mitchison, T. J. Synthesis and phenotypic screening of a guanine-mimetic library. *Chembiochem.* **5**, 1010–1012 (2004).
17. Yarrow, J. C., Feng, Y., Perlman, Z. E., Kirchhausen, T. & Mitchison, T. J. Phenotypic screening of small molecule libraries by high throughput cell imaging. *Comb. Chem. High Throughput. Screen.* **6**, 279–286 (2003).
Highlights the power of cellular imaging in finding active small molecules and shows how compound progression can be carried out to find the molecular target affecting cellular phenotype.
18. Yarrow, J. C., Totsukawa, G., Charras, G. T. & Mitchison, T. J. Screening for cell migration inhibitors via automated microscopy reveals a Rho-kinase inhibitor. *Chem. Biol.* **12**, 385–395 (2005).
19. Ramm, P. *et al.* Automated screening of neurite outgrowth. *J. Biomol. Screen.* **8**, 7–18 (2003).
20. Richards, G. R., Millard, R. M., Leveridge, M., Kerby, J. & Simpson, P. B. Quantitative assays of chemotaxis and chemokinesis for human neural cells. *Assay. Drug Dev. Technol.* **2**, 465–472 (2004).
21. Bahnson, A. *et al.* Automated measurement of cell motility and proliferation. *BMC Cell Biol.* **6**, 19 (2005).
22. Berns, K. *et al.* A large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* **428**, 431–437 (2004).
23. Eggert, U. S. *et al.* Parallel chemical genetic and genome-wide RNAi screens identify cytokinesis inhibitors and targets. *PLoS Biol.* **2**, e379 (2004).
24. Mattheakis, L. C. *et al.* Optical coding of mammalian cells using semiconductor quantum dots. *Anal. Biochem.* **327**, 200–208 (2004).
25. Edwards, B. S., Oprea, T., Prossnitz, E. R. & Sklar, L. A. Flow cytometry for high-throughput, high-content screening. *Curr. Opin. Chem. Biol.* **8**, 392–398 (2004).
26. Irish, J. M. *et al.* Single cell profiling of potentiated phospho-protein networks in cancer cells. *Cell* **118**, 217–228 (2004).
An impressive paper demonstrating how flow cytometry using antiphospho antibodies can provide new ways of clustering cancer-patient populations according to signalling pathways.
27. Morgan, E. *et al.* Cytometric bead array: a multiplexed assay platform with applications in various areas of biology. *Clin. Immunol.* **110**, 252–266 (2004).
28. Wong, C. K., Cheung, P. F., Ip, W. K. & Lam, C. W. Interleukin-25-induced chemokines and interleukin-6 release from eosinophils is mediated by p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and nuclear factor-κB. *Am. J. Respir. Cell Mol. Biol.* **33**, 186–194 (2005).
29. Rausch, O. Use of high-content analysis for compound screening and target selection. *IDrugs* **8**, 573–577 (2005).
30. Ramm, P. Imaging systems in assay screening. *Drug Discov. Today* **4**, 401–410 (1999).
31. Wu, C. C., Reilly, J. F., Young, W. G., Morrison, J. H. & Bloom, F. E. High-throughput morphometric analysis of individual neurons. *Cereb. Cortex* **14**, 543–554 (2004).
32. Burnett, P. *et al.* Fluorescence imaging of electrically stimulated cells. *J. Biomol. Screen.* **8**, 660–667 (2003).
33. Ramm, P. Advanced image analysis systems in cell, molecular and neurobiology applications. *J. Neurosci. Methods* **54**, 131–149 (1994).
34. Takahashi, Y., Sawada, R., Ishibashi, K., Mikuni, S. & Kinjo, M. Analysis of cellular functions by multipoint fluorescence correlation spectroscopy. *Curr. Pharm. Biotechnol.* **6**, 159–165 (2005).
35. Wouters, F. S., Verveer, P. J. & Bastiaens, P. I. Imaging biochemistry inside cells. *Trends Cell Biol.* **11**, 203–211 (2001).
36. Watson, P., Jones, A. T. & Stephens, D. J. Intracellular trafficking pathways and drug delivery: fluorescence imaging of living and fixed cells. *Adv. Drug Deliv. Rev.* **57**, 43–61 (2005).
37. Gasparri, F., Mariani, M., Sola, F. & Galvani, A. Quantification of the proliferation index of human dermal fibroblast cultures with the ArrayScan high-content screening reader. *J. Biomol. Screen.* **9**, 232–243 (2004).
38. Schroeder, K. S. & Neagle, B. D. FLIPR: a new instrument for accurate, high throughput optical screening. *J. Biomol. Screen.* **1**, 75–80 (1996).
Describes the first application of a fluorometric imaging plate reader (FLIPR), one of the most widely adopted cellular imaging tools in the pharmaceutical industry so far.
39. Reynen, P. H., Martin, G. R., Eglen, R. M. & MacLennan, S. J. Characterization of human recombinant α_{2A}-adrenoceptors expressed in Chinese hamster lung cells using intracellular Ca^{2+} changes: evidence for cross-talk between recombinant α_{2A}- and native α_1-adrenoceptors. *Br. J. Pharmacol.* **129**, 1339–1346 (2000).
40. Nickolls, S. A., Fleck, B., Hoare, S. R. & Maki, R. A. Functional selectivity of melanocortin 4 receptor peptide and nonpeptide agonists: evidence for ligand-specific conformational states. *J. Pharmacol. Exp. Ther.* **313**, 1281–1288 (2005).
41. Gopalakrishnan, S. M. *et al.* An offline-addition format for identifying GPCR modulators by screening 384-well mixed compounds in the FLIPR. *J. Biomol. Screen.* **10**, 46–55 (2005).
42. Patel, K. *et al.* Activity of diadenosine polyphosphates at P2Y receptors stably expressed in 1321N1 cells. *Eur. J. Pharmacol.* **430**, 203–210 (2001).
43. Benjamin, E. R. *et al.* State-dependent compound inhibition of $Na_v1.2$ sodium channels using the FLIPR V_m dye: on-target and off-target effects of diverse pharma-cological agents. *J. Biomol. Screen.* **11**, 29–39 (2005).
44. Benjamin, E. R. *et al.* Validation of a fluorescent imaging plate reader membrane potential assay for high-throughput screening of glycine transporter modulators. *J. Biomol. Screen.* **10**, 365–373 (2005).
45. Giuliano, K. A. & Taylor, D. L. Fluorescent-protein biosensors: new tools for drug discovery. *Trends Biotechnol.* **16**, 135–140 (1998).
46. Ghosh, R. N., Grove, L. & Lapets, O. A quantitative cell-based high-content screening assay for the epidermal growth factor receptor-specific activation of mitogen-activated protein kinase. *Assay. Drug Dev. Technol.* **2**, 473–481 (2004).
47. Kapur, R. Fluorescence imaging and engineered biosensors: functional and activity-based sensing using high content screening. *Ann. NY Acad. Sci.* **961**, 196–197 (2002).
48. Grepin, C. *et al.* Increasing the quality of compounds isolated during primary screening: high content screening with Acumen Explorer. *Curr. Drug Discov.* **3**, 37–42 (2003).
49. Jager, S. *et al.* A modular, fully integrated ultra-high-throughput screening system based on confocal fluorescence analysis techniques. *J. Biomol. Screen.* **8**, 648–659 (2003).
50. Oakley, R. H. *et al.* The cellular distribution of fluorescently labeled arrestins provides a robust, sensitive, and universal assay for screening G protein-coupled receptors. *Assay. Drug Dev. Technol.* **1**, 21–30 (2002).
51. Fowler, A., Davies, I. & Norey, C. A multi-modality assay platform for ultra-high throughput screening. *Curr. Pharm. Biotechnol.* **1**, 265–281 (2000).
52. Almholt, D. L. *et al.* Nuclear export inhibitors and kinase inhibitors identified using a MAPK-activated protein kinase 2 redistribution screen. *Assay. Drug Dev. Technol.* **2**, 7–20 (2004).
53. Almholt, K. *et al.* Changes in intracellular cAMP reported by a redistribution assay using a cAMP-dependent protein kinase-green fluorescent protein chimera. *Cell Signal.* **16**, 907–920 (2004).
54. Bertelsen, M. & Sanfridson, A. Inflammatory pathway analysis using a high content screening platform. *Assay. Drug Dev. Technol.* **3**, 261–271 (2005).
Describes the application of cellular imaging to compound profiling by monitoring its efficacy across various signalling pathways.
55. Li, Z. *et al.* Identification of gap junction blockers using automated fluorescence microscopy imaging. *J. Biomol. Screen.* **8**, 489–499 (2003).

56. Lundholt, B. K. *et al.* Identification of Akt pathway inhibitors using redistribution screening on the FLIPR and the IN Cell 3000 analyzer. *J. Biomol. Screen.* **10**, 20–29 (2005).
Describes the use of a HT cellular imaging device to screen compounds and a high-content screening cellular imaging platform to understand the mode of action of a compound.
57. Borchert, K. M. *et al.* High-content screening assay for activators of the Wnt/Fzd pathway in primary human cells. *Assay. Drug Dev. Technol.* **3**, 133–141 (2005).
58. Horrocks, C., Halse, R., Suzuki, R. & Shepherd, P. R. Human cell systems for drug discovery. *Curr. Opin. Drug Discov. Devel.* **6**, 570–575 (2003).
59. Obinata, M. Possible applications of conditionally immortalized tissue cell lines with differentiation functions. *Biochem. Biophys. Res. Commun.* **286**, 667–672 (2001).
60. McNeish, J. Embryonic stem cells in drug discovery. *Nature Rev. Drug Discov.* **3**, 70–80 (2004).
This review highlights the potential power of using stem cells in target discovery and primary screening.
61. Allen, M. *et al.* Deficiency of the stress kinase p38α results in embryonic lethality: characterization of the kinase dependence of stress responses of enzyme-deficient embryonic stem cells. *J. Exp. Med.* **191**, 859–870 (2000).
62. Levenberg, S., Golub, J. S., Amit, M., Itskovitz-Eldor, J. & Langer, R. Endothelial cells derived from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **99**, 4391–4396 (2002).
63. Trounson, A. The production and directed differentiation of human embryonic stem cells. *Endocr. Rev.* 24 Jan 2006 [epub ahead of print].
64. Laschinski, G., Vogel, R. & Spielmann, H. Cytotoxicity test using blastocyst-derived euploid embryonal stem cells: a new approach to *in vitro* teratogenesis screening. *Reprod. Toxicol.* **5**, 57–64 (1991).
65. Mitchell, K. E. *et al.* Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* **21**, 50–60 (2003).
66. Kola, I. & Landis, J. Can the pharmaceutical industry reduce attrition rates? *Nature Rev. Drug Discov.* **3**, 711–715 (2004).
67. Ulrich, R. & Friend, S. H. Toxicogenomics and drug discovery: will new technologies help us produce better drugs? *Nature Rev. Drug Discov.* **1**, 84–88 (2002).
68. Smith, D. A. & van de, W. H. Pharmacokinetics and metabolism in early drug discovery. *Curr. Opin. Chem. Biol.* **3**, 373–378 (1999).
69. Waters, M. D. & Fostel, J. M. Toxicogenomics and systems toxicology: aims and prospects. *Nature Rev. Genet.* **5**, 936–948 (2004).
70. Riley, R. J. & Kenna, J. G. Cellular models for ADMET predictions and evaluation of drug–drug interactions. *Curr. Opin. Drug Discov. Dev.* **7**, 86–99 (2004).
71. Nersesyan, K., Melikyan, G. S. & Stopper, H. Genotoxic activity of newly synthesized derivatives of cyano-pyridone in murine cells *in vivo* and *in vitro*. *Tsitol. Genet.* **38**, 44–48 (2004).
72. Tice, R. R. *et al.* Report from the working group on the *in vivo* mammalian bone marrow chromosomal aberration test. *Mutat. Res.* **312**, 305–312 (1994).
73. Fenech, M. *In vitro* micronucleus technique to predict chemosensitivity. *Methods Mol. Med.* **111**, 3–32 (2005).
74. Fenech, M. The cytokinesis-block micronucleus technique and its application to genotoxicity studies in human populations. *Environ. Health Perspect.* **101** (Suppl. 3), 101–107 (1993).
75. Ekins, S., Nikolsky, Y. & Nikolskaya, T. Techniques: application of systems biology to absorption, distribution, metabolism, excretion and toxicity. *Trends Pharmacol. Sci.* **26**, 202–209 (2005).
76. Pritchard, J. F. *et al.* Making better drugs: decision gates in non-clinical drug development. *Nature Rev. Drug Discov.* **2**, 542–553 (2003).
77. Lesko, L. J. & Atkinson, A. J. Jr. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: criteria, validation, strategies. *Annu. Rev. Pharmacol. Toxicol.* **41**, 347–366 (2001).
78. Frank, R. & Hargreaves, R. Clinical biomarkers in drug discovery and development. *Nature Rev. Drug Discov.* **2**, 566–580 (2003).
79. De Meyer, G. & Shapiro, F. Biomarker development: the road to clinical utility. *Curr. Drug Discov.* **12**, 23–37 (2003).
80. Rolan, P., Atkinson, A. J. Jr & Lesko, L. J. Use of biomarkers from drug discovery through clinical practice: report of the Ninth European Federation of Pharmaceutical Sciences Conference on Optimizing Drug Development. *Clin. Pharmacol. Ther.* **73**, 284–291 (2003).
81. Koop, R. Combinatorial biomarkers: from early toxicology assays to patient population profiling. *Drug Discov. Today* **10**, 781–788 (2005).
82. Nishimura, T. *et al.* Disease proteomics toward bedside reality. *J. Gastroenterol.* **40** (Suppl. 16), 7–13 (2005).
83. Liu, E. T. Expression genomics and drug development: towards predictive pharmacology. *Brief. Funct. Genomic. Proteomic.* **3**, 303–321 (2005).
84. Shibazaki, M., Takeuchi, T., Ahmed, S. & Kikuchia, H. Blockade by SB203580 of *Cyp1a1* induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and the possible mechanism: possible involvement of the p38 mitogen-activated protein kinase pathway in shuttling of Ah receptor overexpressed in COS-7 cells. *Ann. NY Acad. Sci.* **1030**, 275–281 (2004).
85. Traxler, P. *et al.* AEE788: a dual family epidermal growth factor receptor/ErbB2 and vascular endothelial growth factor receptor tyrosine kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res.* **64**, 4931–4941 (2004).
86. Koga, H. *et al.* Elevated levels of VE-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *J. Am. Coll. Cardiol.* **45**, 1622–1630 (2005).
87. Bick, R. J. *et al.* Fluorescence imaging microscopy of cellular markers in ischemic vs non-ischemic cardiomyopathy after left ventricular unloading. *J. Heart Lung Transplant.* **24**, 454–461 (2005).
88. Kelloff, G. J. & Sigman, C. C. New science-based endpoints to accelerate oncology drug development. *Eur. J. Cancer* **41**, 491–501 (2005).
89. Swanson, B. N. Delivery of high-quality biomarker assays. *Dis. Markers* **18**, 47–56 (2002).
90. Colburn, W. A. Biomarkers in drug discovery and development: from target identification through drug marketing. *J. Clin. Pharmacol.* **43**, 329–341 (2003).
91. Rathbun, R. C. Surrogate markers for assessing treatment response in HIV disease. *Ann. Pharmacother.* **27**, 450–455 (1993).
Demonstrates how cellular imaging can be used in the field of clinical biomarkers by monitoring CD4 cell count in patients with HIV.
92. Perlman, Z. E. *et al.* Multidimensional drug profiling by automated microscopy. *Science* **306**, 1194–1198 (2004).
A study that demonstrates the power of cell systems tools when combined with cellular imaging to profile drugs and understand their mechanism of action.
93. Nolan, J. P., Lauer, S., Prossnitz, E. R. & Sklar, L. A. Flow cytometry: a versatile tool for all phases of drug discovery. *Drug Discov. Today* **4**, 173–180 (1999).
94. Asadullah, K., Sterry, W. & Volk, H. D. Analysis of cytokine expression in dermatology. *Arch. Dermatol.* **138**, 1189–1196 (2002).
95. Duramad, P., McMahon, C. W., Hubbard, A., Eskenazi, B. & Holland, N. T. Flow cytometric detection of intracellular TH1/TH2 cytokines using whole blood: validation of immunologic biomarker for use in epidemiologic studies. *Cancer Epidemiol. Biomarkers Prev.* **13**, 1452–1458 (2004).
96. de Weck, A. L. *et al.* Lymphocyte proliferation, lymphokine production, and lymphocyte receptors in ageing and various clinical conditions. *Springer Semin. Immunopathol.* **7**, 273–289 (1984).
97. Lacombe, F. & Belloc, F. Flow cytometry study of cell cycle, apoptosis and drug resistance in acute leukemia. *Hematol. Cell Ther.* **38**, 495–504 (1996).
98. Krutzik, P. O., Irish, J. M., Nolan, G. P. & Perez, O. D. Analysis of protein phosphorylation and cellular signaling events by flow cytometry: techniques and clinical applications. *Clin. Immunol.* **110**, 206–221 (2004).
99. Medintz, I. L., Uyeda, H. T., Goldman, E. R. & Mattoussi, H. Quantum dot bioconjugates for imaging, labelling and sensing. *Nature Mater.* **4**, 435–446 (2005).
100. Rubart, M. Two-photon microscopy of cells and tissue. *Circ. Res.* **95**, 1154–1166 (2004).
101. Paris, S. & Sesboue, R. Metastasis models: the green fluorescent revolution? *Carcinogenesis* **25**, 2285–2292 (2004).
102. Chishima, T. *et al.* Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. *Cancer Res.* **57**, 2042–2047 (1997).
103. Kan, Z. & Liu, T. J. Video microscopy of tumor metastasis: using the green fluorescent protein (GFP) gene as a cancer-cell-labeling system. *Clin. Exp. Metastasis* **17**, 49–55 (1999).
104. Rice, B. W., Cable, M. D. & Nelson, M. B. *In vivo* imaging of light-emitting probes. *J. Biomed. Opt.* **6**, 432–440 (2001).
105. Uhrbom, L., Nerio, E. & Holland, E. C. Dissecting tumor maintenance requirements using bioluminescence imaging of cell proliferation in a mouse glioma model. *Nature Med.* **10**, 1257–1260 (2004).
106. Kumar, S., Kahn, M. A., Dinh, L. & de Vellis, J. NT-3-mediated TrkC receptor activation promotes proliferation and cell survival of rodent progenitor oligodendrocyte cells *in vitro* and *in vivo*. *J. Neurosci. Res.* **54**, 754–765 (1998).
107. Gao, X. *et al. In vivo* molecular and cellular imaging with quantum dots. *Curr. Opin. Biotechnol.* **16**, 63–72 (2005).
108. Michalet, X. *et al.* Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* **307**, 538–544 (2005).
Illustrates the potential of quantum dot beads to track cell fate *in vivo*, and exemplifies the possibility of multiplexing cellular imaging technology.
109. Fischer, H. P. Towards quantitative biology: integration of biological information to elucidate disease pathways and to guide drug discovery. *Biotechnol. Annu. Rev.* **11**, 1–68 (2005).
110. Butcher, E. C. Can cell systems biology rescue drug discovery? *Nature Rev. Drug Discov.* **4**, 461–467 (2005).
111. Sachs, K., Perez, O., Pe'er, D., Lauffenburger, D. A. & Nolan, G. P. Causal protein-signaling networks derived from multiparameter single-cell data. *Science* **308**, 523–529 (2005).
112. Miyawaki, A., Sawano, A. & Kogure, T. Lighting up cells: labelling proteins with fluorophores. *Nature Cell Biol.* (Suppl.), S1–S7 (2003).
113. Zhang, J., Campbell, R. E., Ting, A. Y. & Tsien, R. Y. Creating new fluorescent probes for cell biology. *Nature Rev. Mol. Cell Biol.* **3**, 906–918 (2002).
114. Verkhusha, V. V. & Lukyanov, K. A. The molecular properties and applications of Anthozoa fluorescent proteins and chromoproteins. *Nature Biotechnol.* **22**, 289–296 (2004).
115. Lukyanov, K. A., Chudakov, D. M., Lukyanov, S. & Verkhusha, V. V. Innovation: photoactivatable fluorescent proteins. *Nature Rev. Mol. Cell Biol.* **6**, 885–891 (2005).
116. Hercend, T. *et al.* Immunotherapy with lymphokine-activated natural killer cells and recombinant interleukin-2: a feasibility trial in metastatic renal cell carcinoma. *J. Biol. Response Mod.* **9**, 546–555 (1990).
117. Nagy, R. D. *et al.* Stem cell transplantation as a therapeutic approach to organ failure. *J. Surg. Res.* **129**, 152–160 (2005).
118. Nir, T. & Dor, Y. How to make pancreatic β cells — prospects for cell therapy in diabetes. *Curr. Opin. Biotechnol.* **16**, 524–529 (2005).
119. Keller, G. Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev.* **19**, 1129–1155 (2005).
120. Wollert, K. C. *et al.* Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* **364**, 141–148 (2004).
121. Tang, Y. L. Cellular therapy with autologous skeletal myoblasts for ischemic heart disease and heart failure. *Methods Mol. Med.* **112**, 193–204 (2005).
122. Sykes, M. & Nikolic, B. Treatment of severe autoimmune disease by stem-cell transplantation. *Nature* **435**, 620–627 (2005).
123. Radbruch, A. & Thiel, A. Cell therapy for autoimmune diseases: does it have a future? *Ann. Rheum. Dis.* **63** (Suppl. 2), ii96–ii101 (2004).

124. Mattson, M. P. Emerging neuroprotective strategies for Alzheimer's disease: dietary restriction, telomerase activation, and stem cell therapy. *Exp. Gerontol.* **35**, 489–502 (2000).
125. Tuszynski, M. H. *et al.* A Phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Med.* **11**, 551–555 (2005).
126. Brundin, P. *et al.* Bilateral caudate and putamen grafts of embryonic mesencephalic tissue treated with lazaroids in Parkinson's disease. *Brain* **123**, 1380–1390 (2000).
127. Widner, H. *et al.* Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N. Engl. J. Med.* **327**, 1556–1563 (1992).
128. Bulte, J. W. Hot spot MRI emerges from the background. *Nature Biotechnol.* **23**, 945–946 (2005).
129. Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nature Med.* **4**, 1313–1317 (1998).
130. Zhu, J., Wu, X. & Zhang, H. L. Adult neural stem cell therapy: expansion *in vitro*, tracking *in vivo* and clinical transplantation. *Curr. Drug Targets* **6**, 97–110 (2005).
131. Frangioni, J. V. & Hajjar, R. J. *In vivo* tracking of stem cells for clinical trials in cardiovascular disease. *Circulation* **110**, 3378–3383 (2004).
132. Vogt, A. *et al.* Cell-active dual specificity phosphatase inhibitors identified by high-content screening. *Chem. Biol.* **10**, 733–742 (2003).
133. DiMasi, J. A., Hansen, R. W. & Grabowski, H. G. The price of innovation: new estimates of drug development costs. *J. Health Econ.* **22**, 151–185 (2003).

Acknowledgements
The authors would like to thank C. Waltzinger for her expertise in flow cytometry and C. Johnson-Leger, D. Besson and D. Perrin for critical reading of the manuscript.

Competing interests statement
The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
BACE1 | EGF | MCP1 | MIP1α | PDGF

FURTHER INFORMATION

Molecular Devices imaging products: http://www.moleculardevices.com/pages/instruments/imaging_main.html
Access to this links box is available online.